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IMMUNE RESPONSE IN SCHISTOSOMIASIS.(U)
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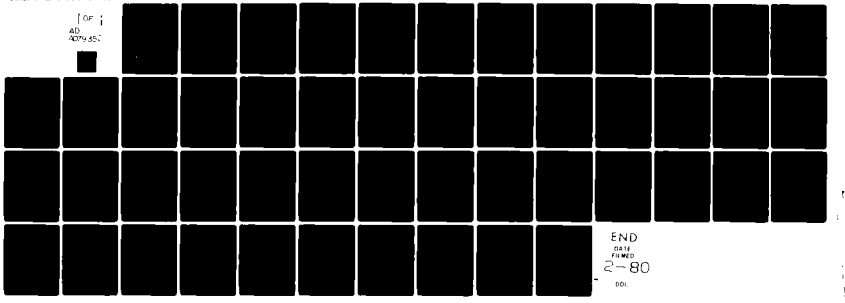

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(6) IMMUNE RESPONSE IN SCHISTOSOMIASIS

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INTRODUCTION

The obsevation was made during the repeated visits to endemic areas of schistosomiasis that many persons residing in these ares for years since birth, do not exhibit any manifestations of the disease. Not only they do not pass schistosomal ova in their urine or stools, but also they do not show any evidence of complications. Nevertheless, it is certain that all these persons were exposed to infection with the parasite. The frequency of exposure to infection may vary,but many are continually exposed ,almost daily. Such exposure is inevitable, as these persons irrigate their land brefooted.They also bathe and drink frm the infected water.

The vast majority of these individuals give history of contracting schistosomiasis usually during childhood or adolescence. History of specific antischistosomal treatment was often lacking. Many received incomplete courses of the antischistosomal drug.

It was assumed therefore that these persons may have leveloped acquired immunity against schistosomes. Such acquired immunity against schistosomiasis has been described in a number of experimental models(Smithers & Terry,1965; Taylor et al.,1973). But the study of this immunity has proved difficult in vivo and in man.

Letter on file

However, the recent development in the understanding of the schistosome cytotoxicity antibody-dependent cell-mediated effector mechanism has provided a means to elaborate an in vitro method to study these antibodies.

It was planned to select and study two groups of these individuals who are apparently immune against schistosomiasis, One group was selected from an endemic area of schist. hematobium, and the other goup from an endemic area of schist. mansoni. The chief aim was to analyse the results and compare them to those obtaine d from a third group with active schistosomiasis and to normal control persons residing in non-endemic areas, and never exposed to infection with schistosomiasis.

Another study was planned to investigate the level of the immune complexes in the sera of patients with schistosomiasis. This aspect of the disease has been so far little studied because of the many difficulties with tests dealing with several kinds of antigens. But the recent development of a number of tests dealing with immune complex has provided a practical means for such study.

The aim of the work was to study any possible correlation between the severity of the infection and the level of the immune complexes. Besides, any possible correlation with the development of complications was also studied.

MATERIAL AND METHODS

CYTOTOXICITY TEST

ANTIBODY-DEPENDENT CELL-MEDIATED DAMAGE TO SCHISTOSOMULAE

Two villages located in the North East of the Nile Delta near Mansoura were chosen for study. The first village (Diarb El Khedr), showed after a general survey of the inhabitants that it is endemic for schistosoma mansoni. The second village(Meet Tarif) was found to be highly endemic in schistosoma hematobium.

Normal control subjects were chosen in Cairo from medical students and laboratory workers who denied history of exposure to infection with schistosomiasis.

All cases were subjected to full clinical examination and laboratory tests, which included :

1.Urine and stools examination for schistosome ova using the centrifugation and salt sedimentation methods respectively.

2.Complete hemogram.

3.Liver fuction tests: serum proteins,serum bilirubin and transaminases.

4.Kidney function tests: blood urea,serum creatinine and complete urine analysis.

5.Blood grouping.

Immunological studies included:

1.Skin testing using schistosome worm antigen and tuberculin purified protein derivative.

2.E rosette test.

The following steps were undertaken for the application of the cytotoxicity test (Butterworth et al.,1977):

1.Parasitic cycle and preparation of schistosomula.

2.Preparation of the antisera.

3.Preparation of the effector cells.

4.Cytotoxicity assay.

Parasite cycle and preparation of schistosomula

A local strain of S. mansoni recovered from patients was maintained by passage in laboratory-bred snails, andguinea pigs.

Schistosomula were prepared by allowing cercariae to penetrate an isolated skin graft from a guinea pig or mouse.

After centrifugation and resuspension in 5 per cent glucose solution, the schistosomula recovered were observed to contain less than than 5 per cent cercariae and intact tails.. The organisms were then stored overnight at 4 degrees C in Hank's balanced salt solution at pH 7.4 containing 0.5 % lactalbumin hydrolysate, 100 U /ml penicillin, and 100 ug/ml streptomycin.Finally, 10% heat-inactivated baboon serum was added to this medium.

Following the overnight storage,the schistosomula were labelled on the next day with ^{51}Cr sodium chromate,obtained from Radiochemical centre,Amersham,England. A dilution of uCi/10 per 1000 organisms was made,followed by incubation for 3 to 4 hours at 37 C. The labelled organisms were next washed four times in Eagle's minimal essential medium (MEM) containing 20 mM Hepes,100 U /ml

streptomycin, and twice in MEM containing heat-inactivated baboon serum. After washing three times, the organisms were resuspended in MEM/heat-ir inactivated baboon serum at the concentration of 500/ml

Preparation of antisera

Serum samples obtained from patients were inactivated at 56 C for one hour before testing for their ability to induce release of chroium from labelled schistosomula

Two dilutions were chosen (1/24 and 1/50) which gave high levels of cell-dependent cytotoxicity.

Preparation and purification of the effector cells
(Unpurified eosinophil cells)

Freh heparinized blood was drawn from patients with known eosinophilia (more than 5 %) : associated with schistosomiasis or other helminthic infections.

5 Vol of blood was allowed to sediment with 1 Vol of 4.5 % dextran in phosphate -buffered saline (Sigma Chemical Co.), for 30 minutes at 37 C .

The supernatant, rich in leukocytes was withdrawn and washed twice in MEM by centrifugation to remove platelets..The pellet rich in eosinophils was next collected and subjected to centrifugation for 40 minutes at 400 g and 4 C over hypaque diluted 1 : 1.7 with distilled water (Mamoud, et al.,1974).

Assay of cytotoxicity

Aliquots of 0.1 ml of schistosomula suspension(500/ml) were placed in Falcon's plastic tubes. Equal volumes of antisera and effector cells were added to each of four replicate tubes.

The tubes were next incubated in humidified airtight boxes at 37 C for 16 hours .

At the end of the incubation period,the contents of each tube were resuspended and centrifugated at 200 g for 5 minutes. One half of the supernatant fluid was withdrawn in a second tube,and both tubes were counted for cromium 51 in a gamma well-type counter(Phillips).

The percentage of isotope release was calculated as follows:

$$\frac{\text{The geometric mean of supernatant x 2}}{\text{The geometric mean of standard}} \times 100$$

where the geometric mean represents the mean of the counts from the four replicates.

STUDY OF IMMUNE COMPLEX

Normal control subjects were selected from medical students, labratory workers and healty persons residing in endemic areas. Persons suspected to have disorders known to be associated with disturbed immunological reactions were eliminated.

Two groups of patients with schistosomiasis were chosen from an endemic area in the Al Mansoura District(north east of the Nile Delta) :

I. Patients presenting with early manifestations of schistosomiasis, and with no cc complications.

II. Patients in late stage of the disease, with complications.

All cases were subjected to full clinical examination and laboratory tests which included:

1. Unrine and stools examination for schistosome ova using the centrifugation and salt sedimentation methods respectively.
2. Counting of schistosome eggs in the urine and stools using the methods of Bradely (1964) and Bell (1963).
3. Complete hemogram.
4. Liver function tests : Serum bilirubin, serum albumin, and transaminases..
5. Kidney function : complete urine analysis, blood urea, serum creatinine..

Immunological studies comprised :

1. Skin tests using schistosome worm antigen and tuberculin purified protein derivative.
2. Tests to demonstrate circulating immune complexes :

1.Method based on thes solubility characteristics of the immune complex namely namely the polyethyleneglycol precipitation method using the optical density measurement.

2Method based on the recognition of the immune complex by specific receptors on the cell surface of the B lymphocytes,namely inhibition of complement-dependent rosette formation (EAC rosette inhibition).

DETECTION OF IMMUNE COMPLEX BY POLYETHYLENEGLYCOL(OPTICAL DENSITY):

5 ml of venous blood were collected in the fasting state,and left to coagulate at room temperature for one hour. The serum was then separated by centrifugation at 3000 rpm for 15 minutes.

The sera were used for the test on the same day of separation in order to avoid the formation of new immune complexes.

The sera were then diluted 1 : 10 with borate buffer (0.1 M-pH 8.4) Equal amounts of the diluted sera were added dropwise to the polyethyleneglycol(PE G

The PEG sera mixtures were next incubated for 2 hours at 4 C.,followed by centrifugation at 4 C,2500 G for 30 minutes.

The supernatant fluid was discarded,and the pellet formed at the bottom of the tube was dissolved in distilled water so that the original volume of the seum used and PEG is replaced.

The turbid solution obtained is again dissolved in 0.1 N sodium hydroxide solution 1: 10.

The resultant solution was read for its optical density in ultra-spectrophotometer w.v 280 mm

EAC ROSETTE INHIBITION ASSAY

The method used was that described by Smith et al.(1975).It consists of the following:

1.Separation of lymphocytes from whole blood by the ficoll-hypaque gradient technique.

2.Sheep erythrocytes (E) were sensitized with antisheep erythrocytes (A), and mice complement (C) by a modification of the method of Borsevach et al.(1972).

Equal volumes of sheep red cells were collected in equal volumes of alzevers solution and stored at 4 C for 48 hours before use.

E cells were prepared by washing the sheep red cells 3 times with sterile PBS pH 7.2 at 150 G for 10 minutes at 4 C.The concentration was adjusted to 0.5% using Hank's medium.

EA were prepared by incubating E cells(0.5%) with equal volume of rabbit antisheep hemolysin (final dilution 1: 6000) for 30 minutes at 37 C. The suspension was washed twice with PBS and resuspended in sterile Hank's medium containing 15% fetal calf serum(inactivated).

For preparation of EAC,a suspension of EA(0.5 %) was incubated with fresh mouse serum as a source of complement (final dilution 1 : 30) for 30 minutes at 37 C. The cells were next washed twice with sterile PBS,and resuspended in sterile minimal essential media or Hank's solution containing 5% inactive fetal calf serum.

Assay of EA and EAC rosettes was done after centrifugation at 200 g for 5 minutes followed by incubation at 37 C for one hour.The cells were then vigorously resuspended,and the number of rosettes were determined.

Eac rosette inhibition was measured by by the incubation of 2×10^6 lymphocytes with 200 ul of test serum and normal serum at 37 C for one hour.

The lymphocytes were washed three times with sterile PBS at pH 7.0, and next incubated with EAC for 30 minutes at 37 C.

This was followed bycentrifugation at 200 g for 5 minutes.The cells were vigorously resuspended,and the number of rosettes were determined.

The inhibition produced by preincubation with a serum was calculated from the formula:

$$100 - \frac{PS}{PC} \times 100$$

where PS = percentage of lymphocytes forming rosettes after incubation with the seum.

and PC =percentage of lymphocytes forming rosettes after incubation with normal seum.

RESULTS

ANTIBODY-CYTOTOXIC STUDY

1185 inhabitants from Meet Tarif and Diarb El Khedr villages were examined for evidence of schistosomiasis by urine and stools testing. Skin testing was used when urine and stools were negative for ova.

The following groups were selected for study:

1. 17 patients with S. hematobium.
2. 30 patients with S. mansoni.
3. 90 persons who appeared immune to schistosomiasis despite frequent exposure to infection.
4. 20 normal control subjects with no previous history or exposure to schistosomiasis.

The results are given in Tables I to XI.

The difference between the mean value of the mansoni group and control was highly significant in both concentrations(1/24 & 1/50). The difference between the hematobium group and control was significant in concentration of 1/24, but insignificant in the concentration of 1/50.

The difference between the mean value of the apparently immue group and normal control persons was highly significant in concentrations of 1/24, but insignificant in concentrations of1/50.

On the contrary, the difference between the apparently immune group and the mansoni group was insignificant in in concentrations of 1/24, but s but significant in concentrations of 1/50.

Table v shows statistical analysis of the values of the cytotoxic test with regard the blood groups. The difference in blood group B is significant.

Table VI shows correlation statistical study between the antibody cytotoxic mean values and the level of eosinophils. A strong correlation was found between the absolute count of ecsinophils in apparently normal persons in concentrations of 1//24 .

IMMUNE COMPLEX STUDY

Sera from 118 patients living in the endemic villages were tested for the presence of immune complex by the hemagglutination rosette inhibition test and the polyethyleneglycol method.

The results are given in Tables VIII to XV.

A significant difference is found between all groups and the normal control except for the hematobium group(early infection), using the hemagglutination rosette inhibition test.

CYTOTOXIC ANTIBODY ACTIVITY IN Table I

NORMAL CONTROL SUBJECTS(NOT RESIDING IN SCHIST. ENDEMIC AREA)

Serial no.	Age	Sex	Percent release chromium 51 1/24	1/50
1	25	M	49.8	40.6
2	22	M	55.5	56.8
3	24	M	59.2	70.6
4	28	M	39.2	55
5	30	M	40.5	45.5
6	28	M	36.3	61.7
7	26	M	51.6	47.4
8	34	M	34.8	64.2
9	32	M	70.6	36.9
10	34	M	67.3	40.2
11	25	F	45.8	32
12	26	M	19	42.4
13	28	M	14.4	30.8
14	35	M	15.2	38.2
15	22	F	20.4	21.8
16	23	F	50.6	35.8
17	25	M	36.3	68
18	32	M	24	65.1
19	30	M	30	27.6
20	26	F	36.6	40.2
		Mean	= 39.9	45.3
		±	=16.52	14.34
		S.E.	= 3.7	3.2

Table II

CYTOTOXIC ANTIBODY ACTIVITY IN PATIENTS WITH SCHIST. HEMATOBIUM (EARLY ACTIVE)

Serial No.	Age	Sex	Percent release cromium 51 1/24	1/50
1	30	M	23.1	45.5
2	35	M	25	47
3	35	M	32.9	39.6
4	22	M	25	37.8
5	23	M	24.6	28.8
6	28	M	35.3	36.3
7	30	M	22.4	41.8
8	10	M	36.2	48.3
9	15	M	30.6	42.9
10	12	M	37.1	49.5
11	23	F	31.5	38
12	12	M	38.7	34.4
13	27	M	34.5	48.7
14	35	F	35	41.3
15	15	M	28.3	39.1
16	37	M	45.9	37.9
17	11	M	22.6	42.5
		Mean =	30.54	42.91
		± =	6.83	5.80
		S.E.(mean)=	1.24	1.16
		p =	0.00125	0.2

Table III

CYTOTOXIC ANTIBODY ACTIVITY IN PATIENTS WITH SCHISTOSOMA MANSONI (EARLY ACTIVE)

Serial No.	Age	Sex	Percent release cromium 51		Blood group
			1/24	1/50	
1	25	F	123.5	64.4	O
2	18	M	53	62.9	A
3	16	M	60.3	71.8	O
4	16	M	63.2	68.5	B
5	30	M	64.2	64.8	AB
6	17	M	132.4	57.7	B
7	15	M	53.1	63.5	A
8	9	M	36.5	62.6	A
9	13	M	62.9	70.8	O
10	19	M	56.7	63.5	A
11	30	M	50.8	48.5	A
12	17	M	44.8	53.1	O
13	14	F	60.5	48.4	A
14	40	M	51.6	54.5	AB
15	14	F	55.6	51.7	A
16	13	F	59.5	48.4	B
17	25	M	69.3	42.3	O
18	17	M	63.3	49	O
19	18	M	56	31.5	A
20	18	M	92.9	101.7	B
21	23	M	81.7	97.6	B
22	20	M	91.7	80.5	O
23	27	M	62	108.8	AB
24	25	M	62.8	90.6	O
25	15	M	71.8	--	B

Table III (Cont.)

Serial No.	Age	Sex	Percent release cromium 51 1/24	1/50	Blood group
26	33	M	80	94.7	O
27	19	M	109.6	--	AB
28	24	M	107.4	90.3	A
29	20	M	74.1	--	B
30	25	M	83.4	65.7	B
		Mean =	71.15	69.92	
		±	=22.93	4.22	
		S.E(mean)=	4.19	3.76	
		p =	o.ooo5	o.ooo5	

Table IV

CYTOTOXIC ANTIBODY ACTIVITY IN SUBJECTS APPARENTLY IMMUNE AGAINST SCHISTOSOMIASIS RESIDING IN ENDEMIC AREA AND EXPOSED TO INFECTION

Serial No.	Age	Sex	History of schist.	WBC	Eosin.	Percent release chromium 51 1/24	1/50	Blood group
1	30	M	+	5800	1	90	61	A
2	26	M	+	6300	2	70.4	49.1	A
3	25	F	+	4700	2	51.1	56	A
4	33	M	+	5700	0	43	22.1	O
5	27	F	+	4400	2	40.1	75.6	B
6	26	M	-	5800	0	54.5	79.4	A
7	55	F	+	5500	0	41.2	60	O
8	30	F	+	6400	1	34.5	51	O
9	25	F	+	5000	3	55	34.5	A
10	30	F	+	9000	2	55.5	35	O
11	55	F	-	6100	1	49.7	60	A
12	22	F	+	6400	2	69	39	B
13	30	F	+	4000	2	42.4	59	B
14	66	M	+	9200	1	96.2	57	A
15	30	M	+	3600	3	97.2	78.2	O
16	22	M	+	4800	5	48.8	40	A
17	47	M	+	8200	4	45	39.5	A
18	50	M	+	8000	1	59.5	49	B

Table IV (Cont.)

Serial No.	Age	Sex	History of Schist	WBC	Eosin.	Percent rela. cromium 51		blood group
19	45	F	+	6000	2	58.5	39	A
20	52	F	-	5600	1	81.6	48	A
21	17	F	+	8000	1	74.6	56.1	A
22	25	F	-	6200	3	77	79.2	)
23	46	M	+	5000	0	58	33	O
24	30	F	+	7200	4	98.6	43.9	B
25	30	M	+	6500	2	77.6	74	A
26	30	M	=	5000	3	60.8	48.8	O
27	55	F	+	6000	3	59.1	76.3	A
28	50	F	-	8200	2	62.7	36.5	A
29	32	M	+	5800	2	64.3	44.2	O
30	29	M	+	6000	3	85.3	35.8	O
31	56	M	+	6500	4	109.1	4[illegible]	A
32	33	M	+	7100	4	89.8	51.2	A
33	25	M	+	&200	1	86.4	54	A
34	32	M	+	7300	1	35.7	47.2	A
35	33	M	+	6000	0	81.1	80.5	A
36	30	M	+	6500	4	74.5	52.7	A
37	28	M	+	4600	1	62	48.1	A
38	40	M	+	5400	1	72	59.3	AB
39	60	F	-	6700	3	76.3	53.1	O
40	55	M	+	5400	0	78	69.8	B

Table IV (Cont.)

Serial No.	Age	Sex	History of schist.	WBC	Eosin.	Percent release chromium 51 1/24	1/50	Blood group
41	15	M	+	7000	2	59.9	74.3	AB
42	30	M	+	7800	3	56.4	63.3	B
43	60	M	+	5800	4	67.6	--	AB
44	40	F	-	6200	5	65.3	85	A
45	27	M	+	5700	2	99	76.7	B
46	60	M	+	5400	5	51.6	45.5	B
47	35	F	+	6000	3	80.8	46.5	B
48	25	M	+	6500	4	56.8	57.5	A
49	50	M	+	4800	+	70.6	37.5	O
50	58	M	+	7000	1	55.5	52.6	AB
51	35	M	+	5500	4	53.5	45.8	O
52	26	M	+	6200	6	56.6	36.8	A
53	49	M	+	7000	1	64	56.6	B
54	35	M	+	7500	4	64.7	48	A
55	42	M	+	7500	2	49.3	68.3	A
56	60	M	+	8500	1	61.6	62.2	A
57	30	M	+	7600	1	72.8	56.8	O
58	15	M	+	6300	4	55.3	52	O
59	55	F	-	6800	4	53.2	49.6	)
60	25	M	+	6600	3	73.9	41.4	O
61	45	M	+	8000	5	81.6	32.4	A
62	18	M	+	6100	5	53.6	42.9	A
63	16	M	+	8000	2	95.9	36.9	O
664	43	M	+	6900	1	54.1	43.8	O

Table IV (Cont.)

Serial No.	Age	Sex	History of schist	WBC	Eosin.	Percent release chromium 51 1/24	1/50	Blood Group
65	22	F	+	7600	3	98.7	82.1	A
66	16	M	+	5200	2	60.9	44.5	A
67	23	M	+	4400	1	82.1	58.8	A
68	50	M	+	5700	3	61.3	69.6	B
69	25	M	+	6100	4	68.7	47.3	O
70	60	M	+	6400	1	84.7	-	O
71	25	M	+	9000	1	49.6	68.6	AB
72	18	M	+	6400	2	97.2	46.8	AB
73	27	M	+	7800	1	53.9	53.	B
74	70	M	-	7300	1	45.5	39.3	O
75	45	F	+	5100	3	53.4	66.2	B
76	23	M	+	5700	6	36	-	A
77	17	M	+	6000	2	81.4	-	O
78	30	M	+	5800	5	61.8	44.5	O
79	16	M	+	7100	0	63.5	43.7	A
80	35	M	+	8000	3	49.5	-	AB
81	22	M	+	6000	3	49	39.4	O
82	16	M	+	8700	2	50	-	ø
83	37	M	+	7600	8	57.5	-	o
84	22	M	+	6300	1	49	-	A
85	20	M	+	6800	6	52	-	A

Table IV (Cont.)

Serial No.	Age	Sex	History of schist.	WBC	Eosin.	Percent release chromium 51 1/24	1/50	Blood group
86	20	M	+	8000	2	81.5	-	B
87	40	M	+	7200	3	50	31.5	O
88	35	M	+	7200	3	69.3	41.3	AB
89	17	M	+	6500	2	109	35	O
90	19	M	+	4600	1	112	69.6	O
				Mean =		66.1	51.3	
				±		=17.8	14.7	
				S.E(mean)=		1.88	1.64	
				p		=0.0005	0.10	

Table V

Statistical analysis of the results of cytotoxicity between the 4 groups

Conc. 1/24	Group		D	Tc	Tt	P
App. Imm.	x	Mansoni	5.2	1.29	1.64	0.10
App. Imm.	xControl		26.1	4.66	1.64	0.0005
App. Imm.	x	Hemat.	35.5	8.52	1.64	0.0005
Mansoni	x	Control	31.7	3.64	1.64	0.0005
Mansoni	x	Hemat.	40.7	7.14	1.64	0.0005
Control	x	Hemat.	9.4	2.18	1.64	0.00125
Conc. 1/50						
App. Imm.	x	Mansoni	31.6	3.72	1.64	0.005
App Imm.	x	Control	5.0	1.36	1.64	0.10
App. Imm	x	Hemat.	8.4	2.27	1.64	0.125
Mansoni	x	Control	18.6	3.59	1.64	0.0005
Mansoni	x	Hemat.	22	4.63	1.64	0.0005
Control	x	Hemat.	5.4	0.91	1.64	0.2

Table VI

Correlation coefficient between the absolute eosinophil count and the mean ofcytotoxicity test (% release of cromium51)

r	=	0.7	(1/24)
r	=	0.01	(1/50)

Table VII

Statistical analysis of the cytotoxicity test in relation to blood groups

Conc. 1/24

Measures	B L O O D G R O U P			
	A	B	AB	O
Mean	64.2	66.9	76.1	63.8
S.D.	15.8	20.63	18.18	17.30
S.E.(mean)	2.67	3.61	4.86	6.53

Conc.1/50

Measures	B L O O D G R O U P			
	A	B	AB	O
Mean	53.3	47.7	53.2	54.1
S.D.	17.4	13.29	11.98	9.09
S.E(mean)	2.98	2.51	3.46	3.71

TABLE VIII

CHIEF CLINICAL AND LABORATORY DATA IN PATIENTS WITH EARLY SCHISTOSOMIASIS

(NO COMPLICATIONS)

Serial No.	Age	Sex	Type of schistos.	Specific treatment	State of Nutrition	Liver function S.Bil. mg	S.Alb. mg.	S.GOPT Units	W B C	Eosinoph. %	Hg %	Skin test(worm antigen) Immediate	Delayed
1	6	M	H	Nil	Fair	0.8	2.8	8	5300	6	73	Neg.	Neg.
2	9	M	H	Nil	Fair	0.7	2.5	11	6900	2	70	Neg	Neg
3	11	M	H	Tartar Em	Moderate	0.7	2.7	23	4800	8	65	Neg.	0.5 cm
4	11	M	H	Nil	Moderate	0.9	3.1	10	6200	4	68	0.8	Neg
5	12	M	H	Nil	Under	0.4	3	8	5850	6	60	Neg	Neg
6	12	M	H	Nil	Moderate	0.7	2.4	14	7200	10	65	Neg	Neg
7	12	M	H	During treatm.	Under	0.5	2.1	32	8120	8	50	Neg	Neg
8	12	M	H	Nil	Moderate	0.7	2.6	9	5000	8	65	--	--
9	12	M	H & M	After	Moderate	0.9	3.1	19	6100	14	65	Neg	Neg
10	13	M	H	During	Good	0.8	2.5	22	4850	7	75	--	--
11	13	F	H	Nil	Under	0.7	2.6	13	3850	8	57	Neg.	Neg
12	13	F	H	Nil	Fair	0.6	2.9	11	9130	2	70	0.9	Neg
13	13	M	M	Nil	Fair	0.4	3.0	9	4200	3	63	Neg	Neg
14	14	F	M	After TE	Moderate	0.7	3.3	15	6100	14	65	--	--
15	14	M	H	Nil	Moderate	0.6	2.8	6	6200	8	60	Neg	Neg

Table
TABLE V_{I}^{II} (Cont.)

Serial No.	Age	Sex	Type of schistos.	Specific treatment	State of Nutrition	Liver Function S.Bil	S.Alb.	S.GOPT	W B C	Eosinph	Hg%	Skin Test Immediate	Delayed
16	14	F	H	After TE	Moderate	0.7	2.7	24	5300	5	63	--	--
17	14	M	H	Nil	Moderate	0.6	2.8	12	5300	8	65	--	--
18	15	F	H	During TE	Good	1.1	3.2	18	6800	8	70	Neg	Neg
19	15	F	H	Nil	Moderate	0.8	2.2	12	5500	5	68	0.8	1.5
20	15	M	H	After Fouadin	Good	0.7	3.0	17	4300	7	70	--	--
21	15	M	H	After Fouad.	Moderate	0.4	2.7	15	5210	4	72	--	--
22	15	M	H	During TE	Good	0.9	2.6	28	6200	5	70	--	--
23	16	M	H	Nil	Moderate	0.9	3.2	6	6200	5	68	09 cm	Neg
24	16	M	H	After TE	Moderate	0.6	2.5	18	8400	14	65	1 cm^2	2 cm2
25	17	M	H&M	After TE	Good	0.6	2.5	35	6120	8	78	--	- -
26	17	M	H	Mid tret.	Moderate	1.1	2.6	22	9200	6	75	Neg	Ng
27	18	M	H	Mid treat.	Moderate	0.9	3.0	42	4200	10	65	0.5	1.2 cm.
28	18	M	H	Mid treat.	Moderate	0.6	2.1	18	5500	7	72	Neg	Ng
29	18	M	H	Nil	Good	0.3	3.2	12	7400	4	80	Neg	Neg
3o	18	M	M	Nil	Moderate	0.5	2.9	14	6500	6	65	--	--

TABLE VIII(Cont)

Serial No.	Age	Sex	Type of schistosom	Specific treatment	State of nutrition	liver function S.Bil	S.Alb.	SGOPT	W B C	eosin	Hg%	Skin test Immediate	Delayed
31	18	M	M	After TE	Moderate	.6	2.8	31	5700	15	55	1.1 cm	Neg
32	18	M	M	After TE	Good	0.9	3.2	33	9500	15	67	0.7 cm	2 m2
33	19	M	H	Nil	Moderate	0.3	3.3	9	9100	4	68	Neg	Neg
34	19	F	H	Nil	Moderate	0.5	2.9	11	5650	7	75	--	--
35	20	F	H	Nil	Good	0.8	3.3	12	5200	3	85	--	--
36	20	M	H	Nil	Good	0.6	2.3	8	3800	8	75	--	--
37	20	M	H	Nil	Moderate	0.3	2.4	7	6500	8	67	Neg	Neg
38	20	M	H	Nil	Moderate	0.8	2.8	11	6400	5	70	Neg	Neg
39	20	M	H	Nil	Moderate	0.3	2.9	25	3800	7	58	--	--
40	20	M	H	Nil	Good	0.7	2.2	5	4250	6	80	--	--
41	20	F	H	Mid tret.	Moderate	1.2	2.9	43	7400	12	72	Neg	Neg
42	21	F	H	Nil	Moderate	0.9	3.1	29	4400	12	68	Neg	Neg
43	21	M	H	Nil	Moderate	0.6	2.7	10	3100	5	65	--	--
44	21	M	H	Nil	Fair	0.7	2.8	8	8400	4	68	--	--
45	21	F	H	Nil	Moderate	0.7	3.5	6	4500	11	70	--	--

TABLE VIII(Cont)

Serial No.	Age	Sex	Type of schistos.	Specific treatment	State of nutrition	Liver function S.Bil.	S.Alb.	SGOPT	W B C	Eosin.	Hg%	Skin Test Immediate	Delayed
46	22	M	H	Nil	Good	0.6	3.3	9	3400	6	77	Neg	Neg
47	22	M	H	Nil	Moderate	0.5	2.9	13	9450	2	75	Neg	Neg
48	23	M	H	Nil	Moderate	1.0	2.2	14	4250	10	72	--	--
49	23	M	H	Nil	Moderate	0.9	2.8	6	3100	7	66	--	--
50	23	F	H	Mid tret.	Moderate	0.4	2.7	45	9800	11	68	--	--
51	23	M	H	Nil	Under	0.7	3.2	10	5600	3	72	Neg	Neg
52	23	M	H	Nil	Moderate	0.6	2.9	22	4700	5	72	--	--
53	23	M	H	Mid tret.	Moderate	0.8	2.5	33	4400	8	75	Neg	Neg
54	24	M	H	Nil	Moderate	0.9	3.1	15	5700	6	65	--	--
55	24	M	H	Nil	Moderate	0.7	2.7	12	3250	5	73	--	--
56	24	M	H	Nil	Moderate	0.7	2.7	4	9450	3	78	--	--
57	25	M	H	Nil	Moderate	0.5	2.5	12	5800	5	70	1.2 cm	2.2
58	25	F	H	Nil	Under	0.6	2.7	11	7300	6	63	Neg	Neg
59	25	M	H	Nil	Moderate	0,6	3.4	6	6600	7	58	--	--
60	25	M	H	Nil	Moderate	0.5	2.8	9	6400	3	59	-- --	--

TABLE VIII(Cont.)

Serial No.	Age	Sex	Type of schistos	Specific treatment	State of nutrition	Liver function S.Bil.	S.Alb.	SGOPT	W.B.C	Eosin.	Hg%	Skin test Immediate	Del
61	25	F	H	Nil	Moderate	0.8	3.0	12	8300	4	62--	--	
62	25	F	H	Nil	Moderate	0.7	2.4	7	5600	3	58	--	--
63	25	M	H	Nil	Good	0.3	2.8	6	2100	5	68	Neg	Neg
64	25M	M	H	Nil	Moderate	0.5	3.0	14	4850	8	68	--	--
65	25	M	H	Nil	Moderate	0.7	2.5	18	3100	7	65	--	--
66	25	M	H	Nil	Moderate	0.7	2.8	11	7350	3	70	Neg	Neg
67	25	M	M	Nil	Under	0.9	2.2	15	7100	5	65	Neg	Neg
68	25	M	H	Nil	Moderate	0.8	2.8	10	6500	4	68	0.7	1.0
69	25	M	H	Mid treat.	Moderate	0.6	2.3	40	4500	11	68	--	--
70	27	M	M	After treat	Good	0.5	2.9	28	3400	9	68	--	--
71	27	F	H	Nil	Moderate	0.8	3.0	14	3300	4	62	--	--
72	27	M	H	Mid treat	Moderate	0.7	2.4	55	6400	7	65	Neg	Neg
73	27	M	H	Nil	Moderate	0.6	2.9	19	5700	9	70	--	--
74	27	M	M	Nil	Moderate	1.1	2.4	17	6380	5	64	--	--
75	28	M	H	Nil	Moderate	0.6	3.0	10	8200	4	75	--	--

TABLE VII (Cont.)

Serial No.	Age	Sex	Type of schistos.	Specific treatment	State of nutrition	Liver function S.Bil.	S.Alb	SGOPT	W B C	EOsin.	Hb%	Skin test Immediate	Del.
76	28	F	H	Nil	Moderate	0.9	3.1	8	3200	4	66	--	--
77	30	M	H	Nil	Moderate	0.7	2.8	12	8750	3	62	--	--
78	32	F	M	After treat	Moderate	0.8	2.3	23	4600	8	58	Neg	Neg
79	33	F	M	After trt	Moderate	0.9	2.9	27	4300	10	63	Neg.	Neg
80	35	M	M	Nil	Under	0.7	2.8	15	6400	6	55	--	--
81	39	M	M	After trt	Moderate	0.7	2.4	8	5200	6	65	0.8	1.8
82	40	F	M	After trt	Under	).8	3.0	29	7100	9	58	--	--
83	45	M	M	Nil	Moderate	0.9	2.6	12	5500	4	72	Neg	Ng

H : Hematobium

M : Mansoni

TE : Tartar emetic

TABLE IX
CHIEF CLINICAL AND LABORATORY DATA IN PATIENTS WITH LATE SCHISTOSOMIASIS

Serial No.	Age	Sex	Type of schistos	Specific trt	State of nutrition	Liver Cm	Spleen Cm	Liver function S.Bil.	S.Alb.	SGOPT	W B C	Eosin.	Hg%	Skin Test Immediate	Delayed
84	10	M	H&M	Nil	Moderate	3	--	0.9	2	22	5800	7	52	Neg	Neg
85	11	M	M	Nil	Under	1.5	3	0.9	1.9	35	13700	3	52	--	--
86	11	M	M	Mid trt	Moderate	--	3	0.9	1.7	62	5100	12	65	--	--
87	13	F	M	After trt	Moderate	--	4	0.7	2	63'0	4800	8	65	Neg	Neg
88	15	M	M	After trt	Moderate	3	5	0.9	1.8	35	6300	7	60	Neg	Neg
89	15	M	M	Nil	Moderate	2	5	1.2	1.3	33	6100	5	67	--	--
90	15	M	M	Nil	Moderate	--	4	1.1	2.2	26	3450	6	56	0.5	1.5 cm
91	16	M	M	Nil	Moderate	4	6	0.9	1.4	55	2300	7	60	Neg	Neg
92	17	M	M	Nil	Under	--	6	0.9	1.3	65	4500	4	65	--	--
93	18	M	M	Nil	Moderate	2	4	0.7	1.9	23	4550	5	67	Neg	Neg
94	18	F	H &M	Nil	Moderate	2	8	0.6	1.1	45	2300	10	60	Neg	Neg
95	19	M	M	Nil	Under	--	5	0.9	1.7	25	6700	5	66	0.7	0.9
96	20	M	M	After trt	Moderate	3	5	0.8	2.0	22	5400	6	60	0.4	1.5
97	20	M	M	Nil	Moderate	3	5	0.6	1.8	33	2800	6	62	--	--
98	22	M	H&M	Nil	Moderate	--	3	0.8	1.9	12	5750	5	57	--	--
99	23	M	M	After trt	moderate	@	%	1.3	2.4	56	6800	7	68	Neg	Neg
100	24	M	M	After trt	Moderate	2	4	0.7	2.1	25	3600	12	65	--	--

TABLE IX (Cont.)

Serial No.	Age	Sex	Type of schistos	Specific trt	State of nutrition	Liver	Spleen	Liver function S.Bil.	S.Alb	SGOPT	W B C	Eosin	Hg%	Skin test Immediate	Delayed
101	25	M	H&M	Nil	Moderate	--	3	0.9	2.6	14	4500	5	66	--	--
102	25	M	M	Mid trt	Moderate	2	3	0.6	1.5	17	9450	6	65	Neg	Neg
103	25	M	M	Mid trt	Moderate	2	4	1.1	2.6	36	12300	5	65	--	--
104	27	F	M	Nil	Under	4	6	0.7	1.7	25	9200	5	58	Neg	Neg
105	27	M	M	Nil	Moderate	1	4	2.6	2.0	86	6700	6	62	--	--
106	28	M	M	Nil	Moderate	2	3	0.7	1.8	20	8500	4	70	--	--
107	30	M	H&M	Nil	Under	--	6	0.8	1.6	32	3300	7	55	--	--
108	30	M	M	Nil	Moderate	--	4	0.9	1.9	15	5600	3	65	--	--
109	30	M	M	Nil	Under	--	10	1.2	1.4	45	3100	7	56	0.5	1.2 cm
110	32	M	M	Nil trt	Moderate	2	4	0.6	15	23	5100	6	60	Neg	Neg
111	32	M	M	Nil	Moderate	--	6	0.7	1.4	26	3800	6	55	--	--
112	35	M	H&M	Mid trt	Moderate	2	5	0.8	2.2	47	4350	11	65	--	--
113	35	F	M	Nil	Moderate	3	6	0.7	1.9	12	7300	6	65	--	--
114	37	M	M	Nil	Under	--	3	0.8	2.1	15	5500	6	59	--	--
115	37	M	M	Nil	Moderate	--	4	0.7	2.1	22	4600	5	63	--	--
116	42	M	M	After trt	Moderate	2	6	0.9	1.9	45	6500	7	60	--	--
117	43	M	M	After trt	Moderate	3	8	0.7	2.4	38	4300	8	65	--	--
118	45	M	M	After trt	Moderate	2	7	0.8	1.8	33	4100	6	55	Neg	Neg

TABLE X

PERCENTAGE OF ROSETTE INHIBITION IN NORMAL CONTROL SUBJECTS

Serial No.	Age	Sex	Percentage
1	9	M	25
2	11	M	0
3	11	M	13
4	12	F	7
5	12	M	10
6	12	F	12
7	13	F	21.7
8	14	M	0
9	14	M	9
10	15	M	0
11	15	M	13
12	16	M	0
13	16	M	5
14	18	F	9
15	18	M	0
16	19	M	0
17	20	M	11.5
18	20	M	8
19	22	M	0
20	23	F	17.4
21	30	M	0
22	35	M	0
23	37	M	34.8
24	40	M	4.3

TABLE .. XI

PERCENTAGE OF ROSETTE INHIBITION IN EARLY CASES

OF SCHISTOSOMIASIS(HEMATOBIUM)

Serial No.	Percentage
3	60
4	0
5	0
6	20
7	45.2
8	60
10	29
11	44
12	23.8
15	57.1
16	0
17	21.4
18	54.7
19	34.7
20	90.5
21	92
22	18.2
23	60
24	13.3
25	12.2
26	80
27	35.7

TABLE XI (cont.)

28	6.6
29	0
33	33.4
34	0
35	0
36	0
37	75
38	27
39	42.8
40	44
41	0
42	0
43	71.4
44	35
45	45
46	70
46	15
47	32
48	42
49	40
50	42
51	84
52	88
53	0

TABLE 1.7 (cont.)

5[illegible]	53.6
5[illegible]	0
56	0
57	0
58	40
60	40
61	0
62	67
63	88
64	0
65	93.3
66	6.6
68	95
69	0
71	27.7
72	35.5
73	59.5

$p = 0.15$

TABLE XII

PERCENTAGE OF ROSETTE INHIBITION IN CASES OF SCHISTOSOMIASIS (MANSONI)

Serial No.	Percentage
5	5
30	54.5
31	0
79	91.3
80	5
81	64
82	10
83	78
84	35
85	32
86	76.2
87	0
88	40
89	36
90	0
91	0
92	73.9
93	16

P = 0.05

TABLE XIII

IMMUNE COMPLEXES IN THE SERA OF NORMAL CONTROL SUBJECTS

Serial No.	Optical Density
1	0.61
2	0.51
3	0.56
4	0.81
5	0.56
6	0.38
7	026
8	0.44
9	0.6
10	0.45
11	0.57
12	0.35
13	0.38
14	0.52
15	0.51
16	0.58
17	0.35
18	0.36
19	0.42
20	0.37
21	0.17
22	o.35
23	0.37
24	0.51

TABLE XIV.

IMMUNE COMPLEXES IN THE SERA OF PATIENTS WITH S.MANSONI & HEMATOBIUM
(EARLY CASES)

Serum No.	Optical Density
9	0.38
13	0.26
14	0.22
18	0.65
19	0.23
23	0.19
24	0.49
25	0.42
27	0.62
28	0.41
30	0.19
35	0.21
67	0.27
77	0.25
79	0.0.3
80	0.20
81	0.17
82	0.48
83	0.61

mean =0.35 s.d = 0.16

p = 0.0005

TABLE XIV

IMMUNE COMPLEXES IN THE SERA OF LATE CASES OF SCHISTOSOMIASIS
(WITH HEPATOSPLENOMEGALY)

Serial No.	Optical Density
84	0.70
85	0.53
86	0.14
87	0.39
88	0.66
89	0.98
92	0.43
98	0.95
104	0.16
106	1.01
109	0.46
113	1.96
114	0.82
115	1.1
116	0.83
117	0.78
118	1.96

Mean = 0.82 S D =0.52

P = 0.005

DISCUSSION

ANTIBODY CYTOTOXICITY TEST

The existence of antibodies cytotoxic for schistosomules in vitro was first reported in monkeys by Clegg and Smithers(1972), and in man by Capron et al.(1973).

Later it was shown by Butterworth (1974) that this in vitro reaction appears to be mediated cooperatively by schistosome specific immunoglobulin G antibodies and granulocytic effector cells whic were identified by later studies as eosinophils (Butterworth,1977),

The results obtained in the present study indicate that eosinophil-dependent antibodies with cytotoxicity against schistosomula are found in a high proportion of patients with known Schist. mansoni infection. On the other hand,the results of patients infected with Scist. hematobium show insignificant variation from the normal

These findings demonstrate that these cytotoxic antibodies are specific to Schist. mansoni infection,which is in agreement with the results of other studies recently reported(Capron, et al.,1977: Butterwort et al,1977: Camus et al. 1977

The results also demonstrate strong correlation to ova countin in the stools Thus patients with egg counts of

60/ml or more were found to show higher levels of serum these eosinophil-dependent cytotoxic antibodies than patients with egg counts less than 60/ml at the time of study .This finding may be of value when used to assess the severity of infection,which is a point of diagnostic importance. Furthermore,it may also prove to be of value in selecting patients for treatment,and in planning the schedule of drug therapy in each individual case.

The results of these cytotoxic antbodies obtained in the present study fro the sera of persons residing in the endemic areas of schistosomiasis ,but apparently immune against the disease are most interesting.These subjects show no clinical manifestation of the disease,though most of them give a history of schistosomiasis. Many of them have received specific treatment,which was usually incomplete.

All these persons were certainly exposed to frequent infections during their work in their farms,bathing in canals or drinking infected water. Yet no one complained of any symptoms suggestive of schistosomisis usually for several years.Their stools and urine were negative for ova.

The level of these cytotoxic antibodies are significantly higher in this group of individuals than normal control subjects not residing in endemic areas.

This finding may suggest that these cytotoxic antibodies persist for a long time in the blood despite the lack of evidence of active infection. They may therefore be considered to play a possible role in building up acquired immunity against reinfection with schistosomiasis.

The predilection to blood group B agrees with previous observations that persons with blood group B are more susceptible to infection(Annual report,1978). But the relationship to the persistence of immunity in some form is obscure.

Similarly, the relationship to eosinophilia is unclear, as patients with higher levels of cytotoxic antibodies show higher degrees of eosinophils in their peripheral blood. The link between these antibodies and eosinophils however is well-established(Glouert & Butterworth,1977; Butterth,1977).

It may be suggested that these individuals have developed immunity against schistosomiasis as a result of multiple reinfections. But it cannot be assumed that this type of cyto toxic antibodies are responsible alone for such immunity. In human schistosomiasis, numerous types of antibodies have already been described(Smithers and Terry,1969). In experimental(Sher et al.,1974,Murrell et al,1975), the production of cytotoxic antibodies by immunization has failed

However, the results derived from in vitro experiments cannot be interpreted directly in terms of resistance to infection. The whole question of acquired immunity in human schistosomiasis is controversial. But, it appears that the demonstration of these cytotoxic antibodiesin individuals exposed almost daily to infection without developing the disease is probably significant. Further work is necessary to elaborate more the nature and influencing factors of this resistance.

DETECTION OF IMMUNE COMPLEX

Immune complexes may be detected by several techniques based on the physicochemical properties of aggregated versus free and native immunoglobulins, such as large molecular weight (Kunket et al.,1961), complement fixation (Agnello et al.,1970;Nydegger et al.,1974; Crangeot and Pillot,1975;Svehag,1975;Hay et al.,1976; Lurhuma et al.,1976), cryoprecipitation (Meltzer and Franklin,1966), exposure of Fc determinants , which are then detected by by the rheumatoid factor (Winchester et al.,1971),and inhibition of lymphoid cells Fc receptors (Theofilopoulos et al.,1976).

These techniques in fact do not directly identify immune complexes, but rather elements generally associated with the presence of immune complexes. Additional shortcomings of these techniques are their insensitivity and high incidence of false positive results, as well as the laborious methodology required.

In the present study, 2 methods were used for the detection of the immune complexes,namely rosette EAC inhibition , and pblyethyleneglycol precipitation method.

These two methods rely on completely different principles. The inhibition of EAC rosette formation is dependent on the presence on the surface of B lymphocytes of receptors for C_{3b} and C_{3d} (Eden et al.,1973).

When these lymphocytes are incubated with sheep erythrocytes sensitized with antibody and coplement, EAC rosettes are formed. If the lymphocytes are preincubated in sera containing complement C3 fixing the immune complex, the receptors for C_{3b} and C_{3d} may be blocked and the formation of EAC rosettes is inhibited.

Polyehyleneglycol method is on the other hand an entirely different (chemical) method. For the purpose of quantitation, it offers a more simple and accurate tehnique.

Polyethyleneglycol(PEG) is a polysaccharide which at a concentration of 20% precipitates most native immunoglobulins, and many other proteins. When the PEG concentration is decreased to 3-5%, the precipitation of immunoglobulins, and many other proteins is significantly decreased, without preventing precipitation of immune complexes formed in vivo or in vitro (Creighton, 1973). The reason why immune complexes precipitate at such low PEG concentration is unknown. It is not necessarily related to the size of of complexes. It may be related to the conformational changes in the immunoglobulins.

The results of both methods suggest that circulating immune complexes are higher in cases showing early infection than in patients in the late stages with complications . Similarly, values are higher in in patients receiving specific antischistosomal treatment, or immediately following treatment.

Values also correlated with the severity of infection as marked by the number of ova passed in urine or stools.

The explanation of these differences is still uncertain. It appears that early infection evokes more antigen or immunoglobulins, or both that become integrated in a complexed form. Similarly, it may be assumed that during or immediately following antischistosomal treatment, antigens may be liberated in excess. This may have a bearing on subsequent course of the disease. More work is necessary in this area.

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